Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2024v45n6p1909

β-glucanase and xylanase for beef cattle on tropical pasture

β-glucanase e xilanase para bovinos de corte em pasto Tropical

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Highlights

β -glucanase and xylanase can be used as an alternative in ruminant nutrition.
β -glucanase and xylanase can increase fiber digestion.
β -glucanase and xylanase can increase nitrogen metabolism.

Abstract

The aim of this study was to assess the impact of administering an energy-protein supplement with fibrolytic enzymes, either singly or in a blend, on the intake, digestibility, ruminal, and metabolic parameters in grazing beef cattle. Five rumen-cannulated Nellore steers, averaging 36 months of age and a body weight of 559.57 ± 35 kg were evaluated using a 5 x 5 Latin Square design. The treatments included a protein-energy supplement (2 g/kg BW) without additives (Control), or supplemented with 4 g β-glucanase/animal (BGLU); 4 g xylanase/animal (XYLA); 4 g β-glucanase and 1 g xylanase/animal (BGLU+XYLA); and 4 g xylanase and 1 g β-glucanase/animal (XYLA+BGLU). The administration of either single fibrolytic enzymes or the enzyme blend did not significantly influence ($P > 0.05$) the intakes of forage dry matter (DM), total DM, crude protein (CP), neutral detergent fiber (NDF), organic matter (OM), digestible OM, or the digestibility coefficients of DM, NDF, CP, and OM. Similarly, the use of these enzymes individually or combined did not impact ($P > 0.05$) the levels of rumen pH, volatile fatty acids, ruminal ammonia nitrogen, microbial nitrogen, serum urea nitrogen, or urinary nitrogen excretion.

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Received: Mar. 11, 2024 Approved: Oct. 29, 2024

Providing fibrolytic enzymes, individually or in blends, does not modify the nutrient intake, digestibility, or metabolism in beef cattle on tropical pastures receiving low levels of protein-energy supplements.
Key words: Digestibility. Ruminants. Supplementation.

Resumo

Objetivou-se avaliar o efeito do fornecimento de suplemento proteico energético contendo enzimas fibrolíticas em forma individual ou em uma combinação de enzimas sobre o consumo, digestibilidade parâmetros ruminais e metabólicos de bovinos de corte a pasto. Foram utilizados cinco novilhos Nelore canulados no rúmen, com idade média de 36 meses e peso corporal (PC) de 559,57 ± 35 kg em delineamento Quadrado Latino 5 x 5. Os tratamentos avaliados foram o fornecimento de suplemento (2 g/kg de PC) proteico-energético sem a adição de aditivos (Controle), ou com a adição de 4 g/animal beta-glucanase (BGLU), adição de xilanase (XILA, 4g/animal), adição de beta-glucanase e xilanase (BGLU+XILA, 4g de beta-glucanase/animal + 1g de xilanase/animal) e a adição de 4g de xilanase/animal de xilanase e 1g de beta-glucanase/animal beta-glucanase (XILA+BGLU). O fornecimento de enzimas fibroliticas individuais ou blend enzimático não afetou (P > 0,05) o consumo de matéria seca de forragem, matéria seca (MS), proteína bruta (PB), fibra em detergente neutro (FDN), matéria orgânica (MO), matéria orgânica digestível e os coeficientes de digestibilidade de MS, FDN, PB e MO. As enzimas fibroliticas individuais e blend enzimático não influenciaram nas concentrações (P > 0.05) do pH ruminal, ácidos graxos voláteis, nitrogênio amoniacal ruminal, nitrogênio microbiano e nitrogênio ureico sérico e excreção de nitrogênio urinário. O fornecimento de enzimas fibrolíticas individualmente ou em blend enzimático não altera o consumo, digestibilidade e o metabolismo de nutrientes para bovinos de corte mantidos em pasto tropical recebendo suplemento proteico energético em baixas quantidades.
Palavras-chave: Digestibilidade. Ruminantes. Suplementação.

Introduction

In tropical areas, the majority of beef cattle are raised on pasture (Poppi et al., 2018). However, the high lignification of tropical forage cell walls poses a challenge to digestibility (Azevedo et al., 2014). Incorporating fibrolytic enzymes into the diets of ruminants could enhance the digestibility of diets for pasture-fed animals.

Exogenous fibrolytic enzymes can improve microbial adhesion and colonization on feed particles and alter digesta viscosity (Beauchemin et al., 2019), potentially leading to enhanced fiber digestion and feed utilization (Arriola et al., 2017). Given the high cell wall content of tropical forages and the presence of xylans and β-glucans in these walls, supplying β-glucanase and xylanase enzymes is a potential strategy to improve forage utilization (Cevallos et al., 2006). Nevertheless, the effectiveness of these enzymes in ruminants remains debated.

Most studies on fibrolytic enzymes in cattle have been conducted in feedlots, where high concentrate ratios alter the ruminal environment, decreasing pH and increasing temperature, conditions that favor

the activity of exogenous fibrolytic enzymes (Mendoza et al., 2014). Data on the use of these enzymes in pasture-grazing beef cattle are limited. Grazing cattle typically have a near-neutral ruminal pH, which may not support fibrolytic enzyme activity (McAllister et al., 2001). However, concentrate supplementation can change fermentable substrates, causing a fluctuation in the rumen microbial population and potentially reducing fiber utilization. In this way, fibrolytic enzymes might thus stabilize substrates and improve fiber digestion.

We thus hypothesize that fibrolytic enzymes would increase the digestibility of the fibrous fraction in the diets of pasture-fed cattle, and that using enzyme mixtures would further enhance fiber digestion. Therefore, the aim of this study was to investigate the effects of providing an energy-protein supplement with fibrolytic enzymes, either individually or in blends, on the intake, digestibility, ruminal, and metabolic parameters of beef cattle on pasture.

Material and Methods

All experimental activities received approval from the Ethics and Animal Welfare Committee of DSM/TORTUGA (CEUA/DSM approval no. BR 181203).

Study location and animals

The research was conducted at the DSM/Tortuga Center for Innovation and Applied Ruminant Science, situated in Rio Brilhante-MS, Brazil (21°36'23" S 54°47'38" W). Five rumen-cannulated Nellore steers, each 36 months old and averaging a body weight (BW) of 559.57 ± 35 kg, were used in a 5 x 5 Latin Square design. All steers were housed in a single 2-ha paddock, planted with Brachiaria brizantha cv. Xaraés.

Treatments

The study involved five treatment protocols: a protein-energy supplement at 2 g/kg of BW without additives (Control); the supplement plus 4 g β-glucanase/animal (BGLU); the supplement plus 4 g xylanase/animal (XYLA); the supplement plus 4 g β-glucanase and 1 g xylanase/animal (BGLU+XYLA); and the supplement plus 4 g xylanase and 1 g β-glucanase/animal (XYLA+BGLU).

The animals were transferred daily to a management corral at 10h00 for concentrate supplement administration through a ruminal cannula. Enzymes were mixed into the supplement immediately before administration. Table 1 describes the chemical composition of the supplement (consisting of ground corn, soybean meal, urea, and minerals) and pasture.

Table 1
Chemical composition (g/kg DM) of supplement and forage

	Supplement	Forage
Dry matter	850.0	300.8±3.17
Organic matter	935.5	920.0±0.90
Crude protein	250.0	80.1±1.24
Neutral detergent fiber	197.1	700.6±1.70

Animal management and pasture assessment

Each experimental period lasted 21 days, characterized by the following phases: adaptation to the supplement from day 1 to 10; external marker application from day 11 to 19; feces and urine collection three times daily (06h00, 12h00, and 18h00) from day 16 to 19; and rumen fluid sampling at 10h00 and 14h00 on days 20 and 21.

Forage mass (kg DM/ha) was measured every 21 days by clipping four areas of a quadrat (0.25 m^2) randomly selected within the paddock to 1 cm above the ground. The chemical composition of the consumed forage was assessed at the start and every 15 days thereafter through manual grazing simulation.

Nutrient intake and digestibility

To estimate fecal excretion, titanium dioxide was administered (20 g/animal/day) via the ruminal cannula inside paper cartridges (10 g at 06h00 and 10 g at 17h00). Forage intake was assessed using the internal marker indigestible neutral detergent fiber (iNDF).

Fecal samples were collected promptly following spontaneous defecation, packed in plastic bags, and stored at -20 °C for subsequent analysis. Urine samples were collected after spontaneous urination, diluted with H_2SO_4 (0.036N) in a 1:4 ratio (urine: H_2SO_4), and preserved at -20 °C for future measurements of nitrogen, creatinine, allantoin, and uric acid levels.

Rumen fluid was manually obtained at the liquid-solid interface within the rumen and strained through gauze. Rumen pH was immediately measured using a portable pH meter (TEC-3P-MP, Tecnal, Piracicaba, SP, Brazil). Following pH measurement, a 25-mL aliquot was acidified with 0.5 mL of H_2SO_4 (1:1) and stored at -20 °C for future rumen ammonia nitrogen analysis. Additionally, a 1.2-mL sample was treated with 0.3 mL of 25% metaphosphoric acid for subsequent volatile fatty acid (VFA) concentration analysis in the rumen.

Blood samples were taken from the caudal vein using vacuum tubes, centrifuged at 3,000 xg for 15 min, and the serum was stored at -20 °C for later urea concentration analysis.

Fecal excretion (kg/day) was determined as the ratio of the supplied titanium dioxide (g/day) to its fecal concentration (g/kg). Forage dry matter intake (FDMI) was estimated with iNDF as the internal marker, using the formula:

$$FDMI = \frac{[(FE \times iNDF_{feces}) - iNDF_{supplement}]}{iNDF_{forage}} + SDMI$$

where FE = fecal excretion (kg/d); $iNDF_{feces}$ = concentration of iNDF in feces (kg/kg); $iNDF_{supplement}$ = amount of iNDF in the supplement (kg); $iNDF_{forage}$ = concentration of iNDF in the forage (kg/kg); and SDMI = supplement dry matter intake.

The daily urine volume was estimated from the daily creatinine excretion and its concentration in spot samples, as described by Silva et al. (2012).

Laboratory analyses

The concentrations of creatinine and uric acid in urine were measured using commercial kits (Analisa®, Belo Horizonte, MG, Brazil). Allantoin concentrations were assessed through liquid chromatography, adhering to the procedure proposed by George et al. (2006). Urinary nitrogen excretion was quantified employing the Kjeldahl method, as previously described.

Rumen ammonia nitrogen levels (RAN) were analyzed following the modified Chaney and Marbach (1962) method, where phenol was replaced with a 12% sodium salicylate solution, as suggested by Felix and Cardoso (2004). Volatile fatty acids (VFA) were measured based on the method by Erwin et al. (1961), utilizing a gas chromatograph (Model 370) equipped with a Chromosorb 101 column, 15% Tween 80, and 1.5 g of phosphoric acid. Serum urea concentrations were determined using a commercial kit (Gold Analisa, Belo Horizonte, MG, Brazil).

Samples of forage, feces, and supplements were oven-dried at 55 °C for 72 h and ground using a Wiley mill with 1 and 2-mm sieves. These samples were then analyzed for dry matter (DM) (method no. 920.39), crude protein (CP) (method no. 954.01), and organic matter (OM) (method no. 942.05), in accordance with AOAC (1990) guidelines. Neutral detergent fiber (NDF) was assessed as per Mertens (2002), employing thermostable α-amylase. Indigestible neutral detergent fiber (iNDF) was quantified after a 288-h *in situ* incubation using fiber analysis bags (F57, Ankom®), following the method described by Valente et al. (2011). Titanium dioxide concentrations were determined using the colorimetric method outlined by Titgemeyer et al. (2001).

Statistical analysis

Data were analyzed using the MIXED procedure of SAS (Statistical Analysis System, version 9.0), within a 5 x 5 Latin square design, employing the following statistical model:

$$Y_{ijk} = \mu + T_i + A_j + P_k + e_{ijk}$$

where Y_{ijk} = dependent variable; μ = overall mean; T_i = treatment effect; A_j = animal effect; P_k = period effect; e_{ijk} = error associated with each observation, assuming a normal and independent distribution of the data.

All means were subjected to analysis of variance and compared using Fisher's LSD test, with a significance level set at 5%.

Results and Discussion

Forage allowance averaged 5,615 kg DM/ha, with a stocking rate of 3.6 AU, leading to a forage supply of 3.94 kg of forage/kg of BW. This finding suggests that forage intake was not constrained by forage allowance. As per the findings of Greenwood et al. (2017), a forage allowance below 1,800 kg DM/ha may restrict forage intake. The average protein content of the forage was determined to be 8.06 ± 1.24% (Table 1).

The average quantity of supplement provided was 1.26 kg/animal/day (Table 2). The administration of β-glucanase and xylanase, either individually or in combination, did not significantly influence (P > 0.05) the intakes of FDM, TDM, CP, NDF, OM, or DOM. Likewise, the digestibility of DM, OM, CP, and NDF remained unchanged (P > 0.05) with the inclusion of fibrolytic enzymes (Table 2).

Table 2
Effect of fibrolytic enzyme addition on the intake and digestibility of grazing steers

	Control	Beta	Xyla	Beta+Xyla	Xyla+Beta	SEM	P-value
Intake, kg/steer							
FDM	8.83	9.57	9.09	8.40	8.81	0.36	0.17
SDM	1.20	1.28	1.33	1.24	1.25	0.47	0.44
TDM	10.06	10.83	10.41	9.69	10.03	0.35	0.17
CP	1.08	1.15	1.14	1.06	1.08	0.03	0.23
NDF	6.47	7.00	6.66	6.17	6.45	0.24	0.17
OM	9.28	9.99	9.61	8.94	9.25	0.41	0.18
DOM	6.64	7.00	6.75	6.23	6.54	0.21	0.18
Digestibility, %							
DM	68.54	67.07	66.98	66.38	67.53	1.40	0.11
CP	70.96	68.58	70.11	68.72	68.90	3.55	0.26
NDF	66.32	66.55	66.94	67.15	67.15	0.92	0.95
OM	71.60	70.20	70.10	69.60	70.71	1.13	0.10

(Control) Without the addition of enzymes; (Beta) Addition of β-glucanase; (Xyl) Addition of xylanase; (Beta+Xyl) Addition of β-glucanase + xylanase; (Xyl+Beta) Addition of xylanase + β-glucanase. (FDM) Forage dry matter; (SDM) Supplement dry matter; (TDM) Total dry matter; (CP) Crude protein; (NDF) Neutral detergent fiber; (OM) Organic matter; (DOM) Digestible organic matter.

Previous research on ruminants has explored the effects of providing fibrolytic enzymes either singly (Kondratovich et al., 2019; Ran et al., 2019) or in various combinations (Colombatto et al., 2003; Song et al., 2018). Some studies (Gandra et al., 2017; Pinos-Rodríguez et al., 2002) have suggested that the combined administration of these enzymes would be responsible for changes in nutrient intake and digestibility. However, supporting the findings of this study in grazing animals, Kondratovich et

al. (2019) and Ran et al. (2019) reported no significant effects from adding fibrolytic enzymes to the diets of feedlot cattle fed low-quality roughages.

Most existing studies were conducted on feedlot animals, with inconsistent responses (Ran et al., 2019). There is a scarcity of research on the use of fibrolytic enzymes in beef cattle grazed on tropical pastures. The interactions between cattle and pasture make this production system challenging to comprehend. In the rumen, fibrolytic bacteria are primary producers of fibrolytic enzymes. Dietary nutrient changes can limit their growth, potentially reducing enzyme production due to competition among rumen bacteria for substrates (Rabee et al., 2022). The supplementation of concentrate to pasture-grazed cattle exemplifies such competition for substrates. Introducing concentrate supplements could lead to fluctuations in the microbial population in the rumen, thereby affecting enzyme production. However, the relatively small amount of concentrate supplement provided, in proportion to the size of the steers, might not have adversely affected the growth of fibrolytic bacteria and, consequently, the ruminal enzyme production, minimizing the impact of adding exogenous enzymes due to the lack of prior enzyme limitation.

C4 grasses are typically considered low-quality feed due to their high cell wall content (65-75%) and low digestibility (45-55%) (Cevallos et al., 2007; Freiria et al., 2018). Beta-glucans, constituents of the cell wall, are prevalent in both fresh and preserved forages as well as grains (Cherdthong et al., 2018). When cattle consume diets with higher proportions of such components, ruminal digestion could be compromised, and nutrient absorption in the small intestine might be hindered by increased dietary viscosity (Hristov et al., 2000). Consequently, administering β-glucanase could enhance the passage rate and facilitate nutrient absorption (McAllister et al., 2001). Cherdthong et al. (2018) noted an increase in nutrient intake and digestibility in beef cattle supplemented with β-glucanase and fed rice straw. However, tropical grasses have low levels of β-glucan (Bobade et al., 2022), indicating no enzymatic limitation for the degradation of this component in the rumen of grazing cattle.

Among the constituents of the plant cell wall, hemicellulose is the fastest fermenting component in the rumen (Soltan et al., 2013). facilitate includes xylans (Mendoza et al., 2014). Under normal conditions, ruminal microorganisms efficiently produce xylanase, facilitating dietary fiber breakdown. Yet, several studies have indicated that exogenous xylanase supplementation can enhance fiber degradation, particularly in diets high in concentrate supplements. These supplements tend to acidify rumen pH, diminishing the activity of fiber-degrading ruminal microorganisms.

As stated by Van Soest (1994), fiber degradation is impeded when rumen pH falls below six. Thus, in such scenarios, the addition of fibrolytic enzymes might have a more significant proportional impact. In our research, the inclusion of exogenous enzymes did not significantly alter ($P > 0.05$) the measurements of pH, VFA, RAN, or MICN (Table 3). The mean rumen pH was observed at 6.94, with average concentrations of acetate, propionate, and butyrate at 74.9, 17.5, and 16.9 mmol, respectively, and RAN at an average of 14.41 mg/dL.

Table 3
Effect of adding fibrolytic enzymes on ruminal parameters and nitrogen metabolism

	Control	Beta	Xyla	Beta+Xyla	Xyla+Beta	SEM	P value
SDM	1.20	1.28	1.33	1.24	1.25	0.47	0.44
TDM	10.06	10.83	10.41	9.69	10.03	0.35	0.17
CP	1.08	1.15	1.14	1.06	1.08	0.03	0.23
NDF	6.47	7.00	6.66	6.17	6.45	0.24	0.17
OM	9.28	9.99	9.61	8.94	9.25	0.41	0.18
DOM	6.64	7.00	6.75	6.23	6.54	0.21	0.18
DM	68.54	67.07	66.98	66.38	67.53	1.40	0.11
CP	70.96	68.58	70.11	68.72	68.90	3.55	0.26
NDF	66.32	66.55	66.94	67.15	67.15	0.92	0.95
OM	71.60	70.20	70.10	69.60	70.71	1.13	0.10

(Control) Without the addition of enzymes; (Beta) Addition of β-glucanase; (Xyl) Addition of xylanase; (Beta+Xyl) Addition of β-glucanase + xylanase; (Xyl+Beta) Addition of xylanase + β-glucanase. (RAN) Rumen ammonia nitrogen; (VFA) Volatile fatty acids; (SUN) Serum urea nitrogen; (UNE) Urinary nitrogen excretion; (MICN) Microbial nitrogen.

The administration of fibrolytic enzymes did not significantly affect ($P > 0.05$) the levels of SUN or UNE (Table 3). The average SUN was recorded at 14.02 mg/dL, and UNE at 92.16 g/day. Exogenous fibrolytic enzymes are most active at a pH range of 4 to 5 and at temperatures around 50 °C (Adesogan et al., 2017). The absence of these optimal conditions in the rumen may have constrained their action. Such conditions are seldom encountered in the rumen of grazing cattle. In our study, the rumen pH was maintained near neutrality, failing to provide the optimal pH and temperature required for the activity of exogenous fibrolytic enzymes, thereby diminishing their activity. The inability to form a stable and highly active enzyme-substrate complex might have restricted enzymatic action, leading to unchanged ruminal degradation, as indicated by the lack of alterations in RAN, VFA, and MICN.

Conclusion

The supplementation of β-glucanase and xylanase enzymes does not impact nutrient intake, digestibility, or metabolism in beef cattle grazing on tropical pastures and receiving low levels of protein-energy supplements.

Acknowledgment

We would like to thank DSM (Produtos Nutricionais SA, São Paulo, Brazil) for funding this project.

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